

002227-25044660

FIGURE 1

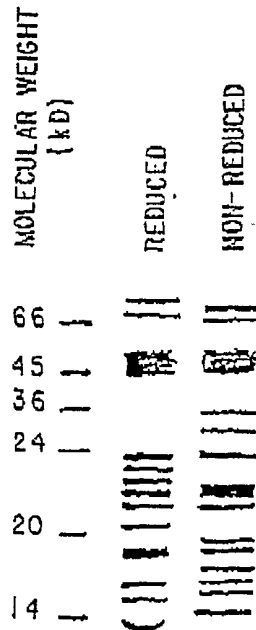


FIGURE 2

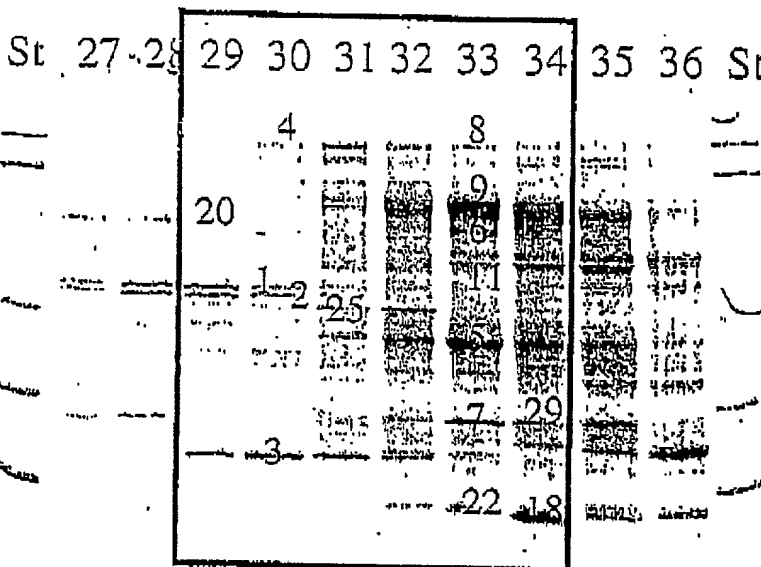
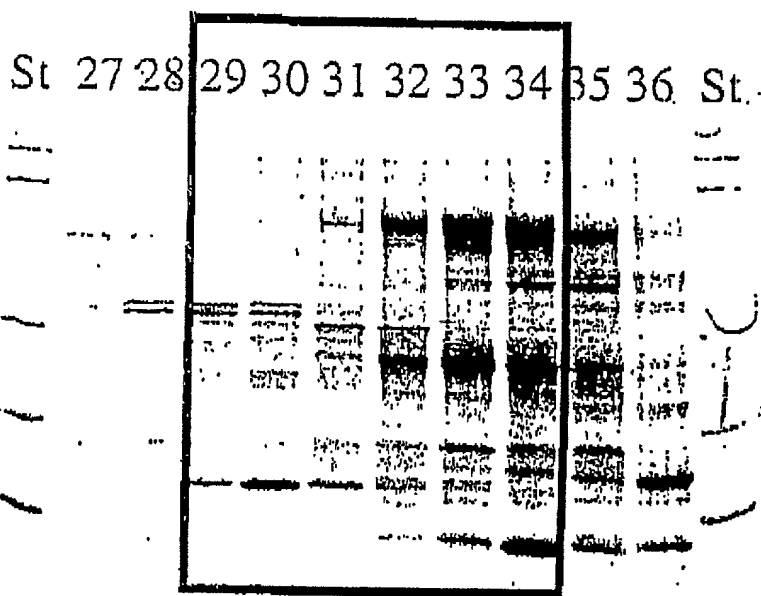
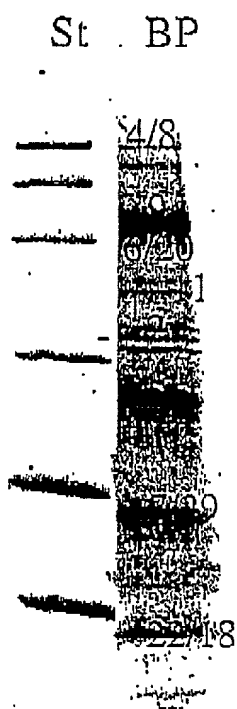


FIGURE 3



Band No.	Identity
1	histone H1.c
2	histone_H1.c
3	ribosomal protein RS20
4	similar to ribosomal protein LORP
5	BMP-3
6	α 2 macroglobulin RAP & BMP-3
7	similar to ribosomal protein LORP
8	BMP-3
9	BMP-3
11	ribosomal protein RL6 & BMP-3
18	TGF- β 2/SPP24
20	Factor H
22	TGF- β 2
25	BMP-3 & H1.x
29	BMP-3 & ribosomal protein RL32

FIGURE 4

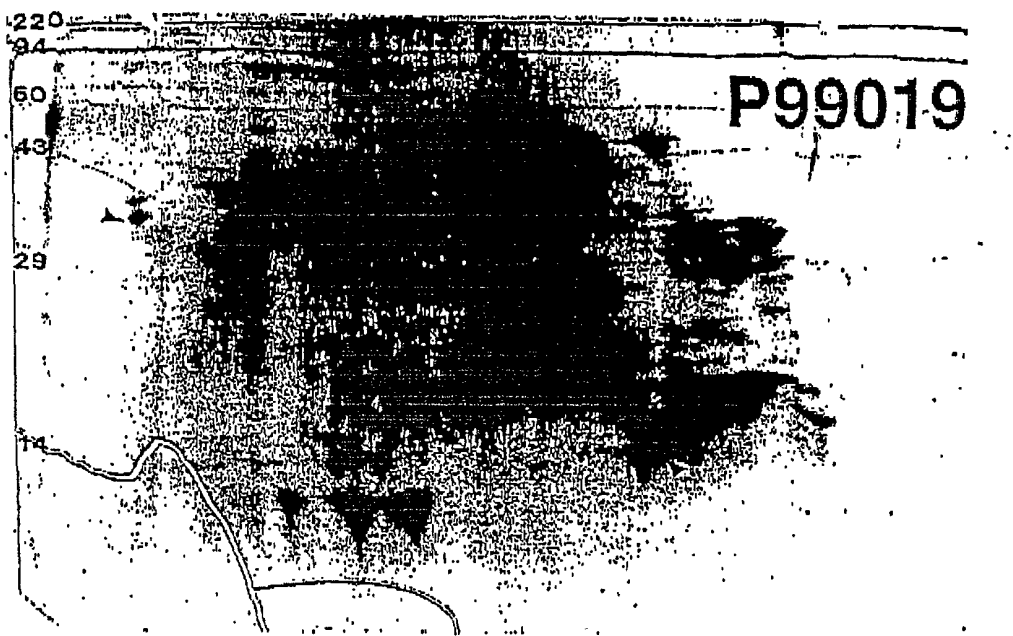
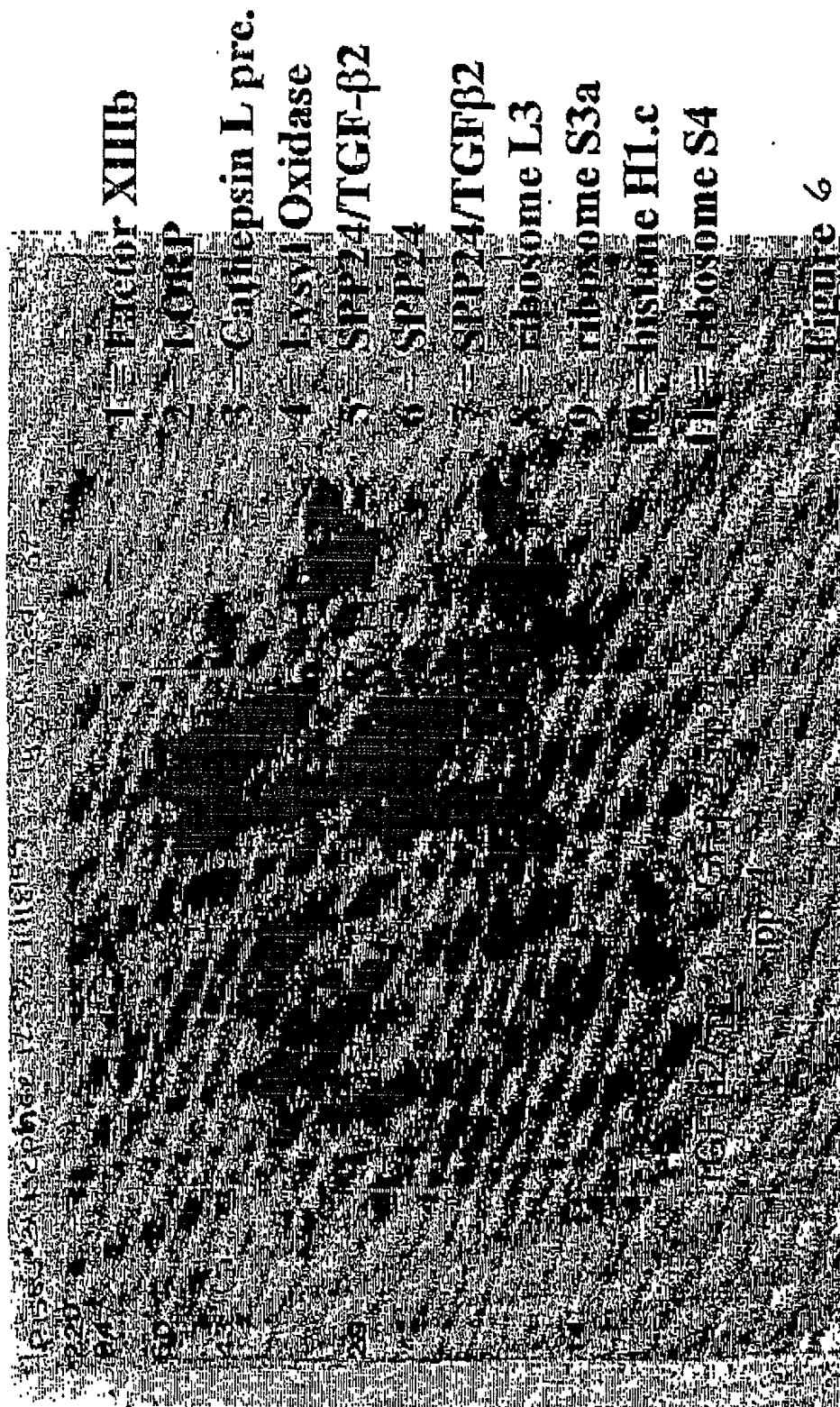


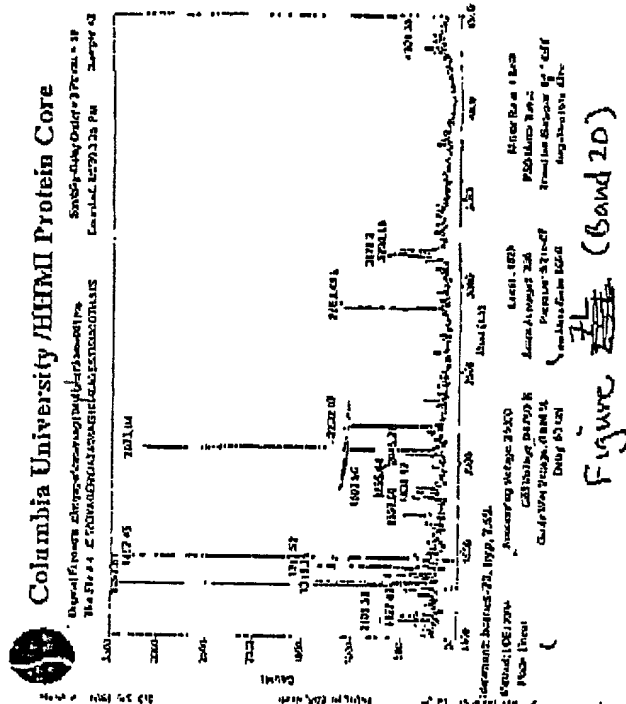
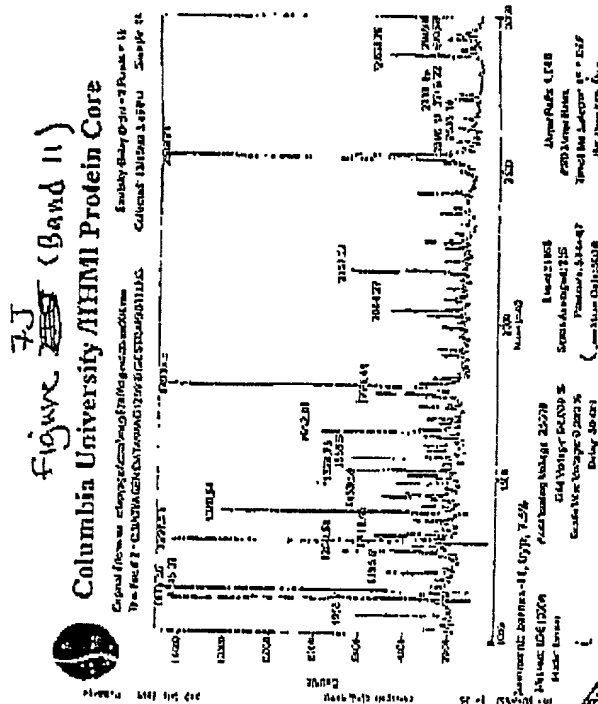
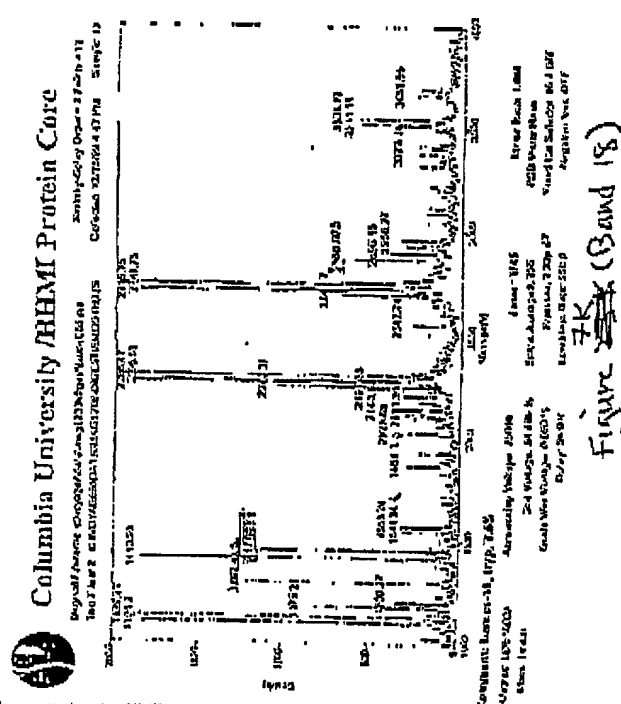
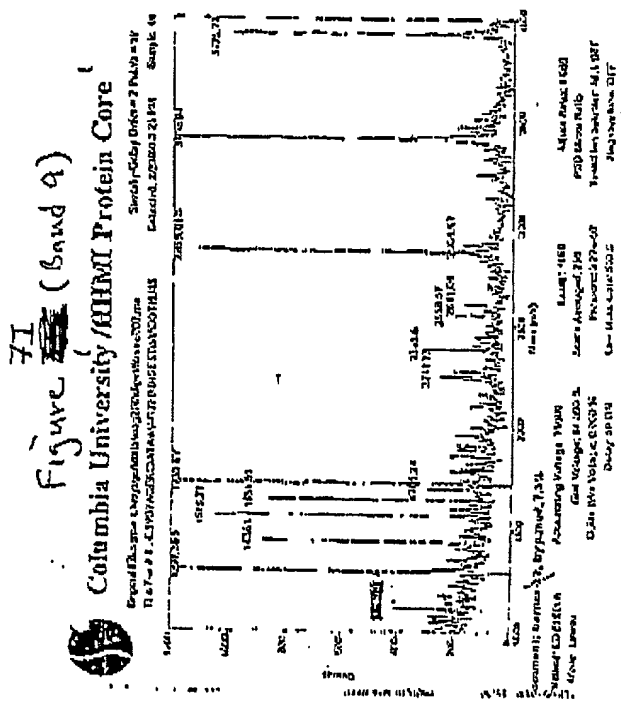
FIGURE 5



P, 05/08

FAX NO. 3039643101

APR-07-00, FRI 12:54 PM SULZER ORTHO. BIOLOGICS



7M ~~Figure~~ (Band 22)

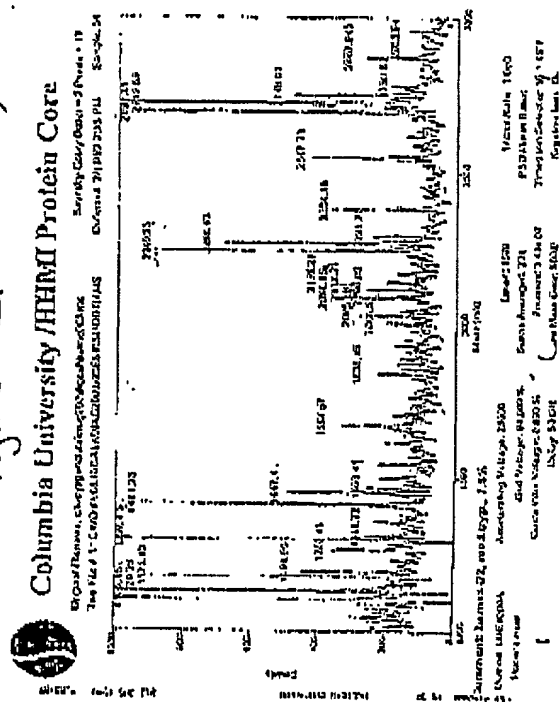
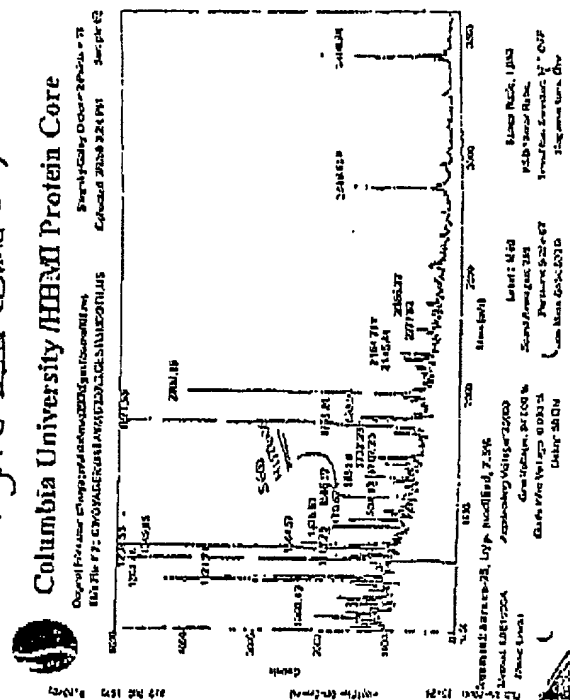


Figure ~~25~~ (Band 25)



Columbia University AHMM Protein Core

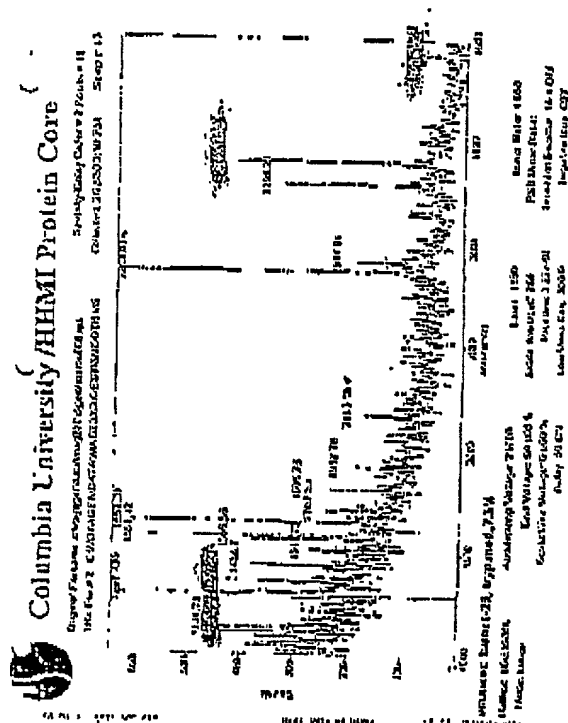


Figure ~~70~~ (Band 29)

00227-804460

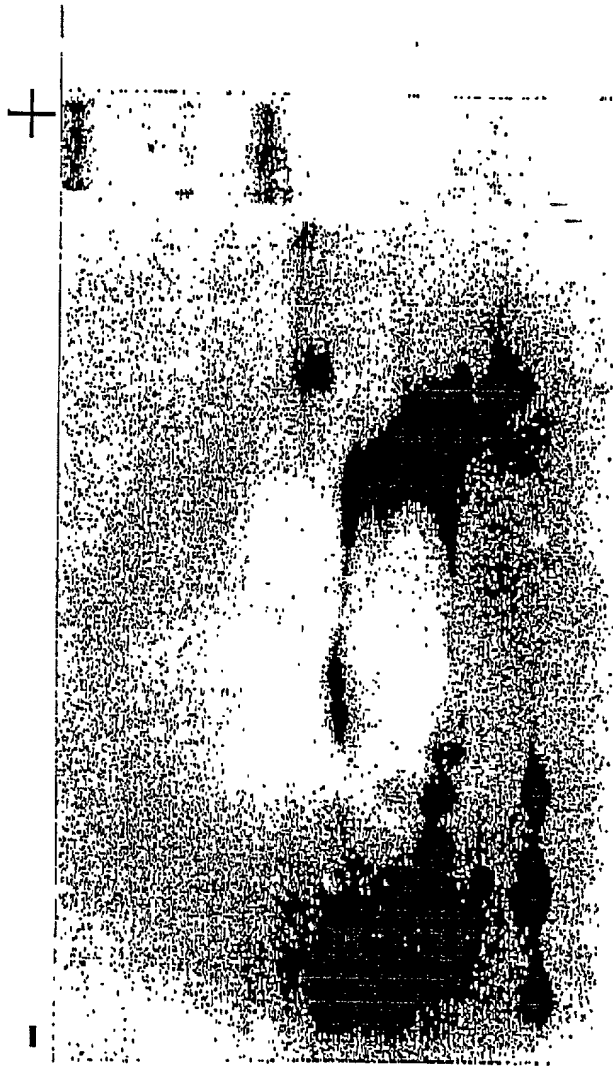


FIGURE 8

h2

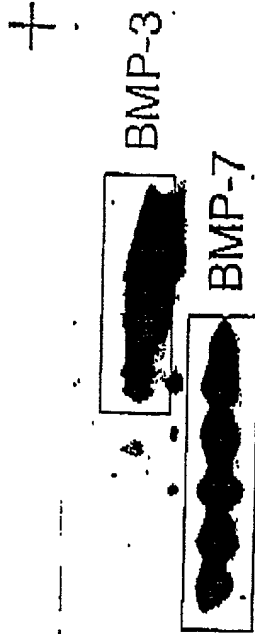


FIGURE 9A

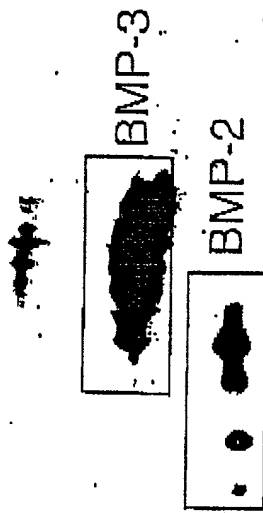


FIGURE 9B

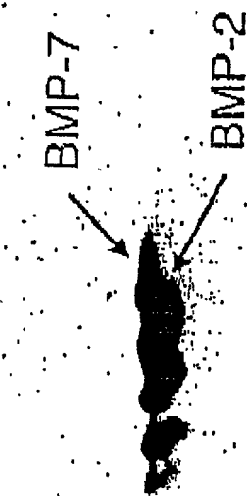


FIGURE 9C



FIGURE 9D

002227-8004460

58

002221-8604460

FIGURE 10

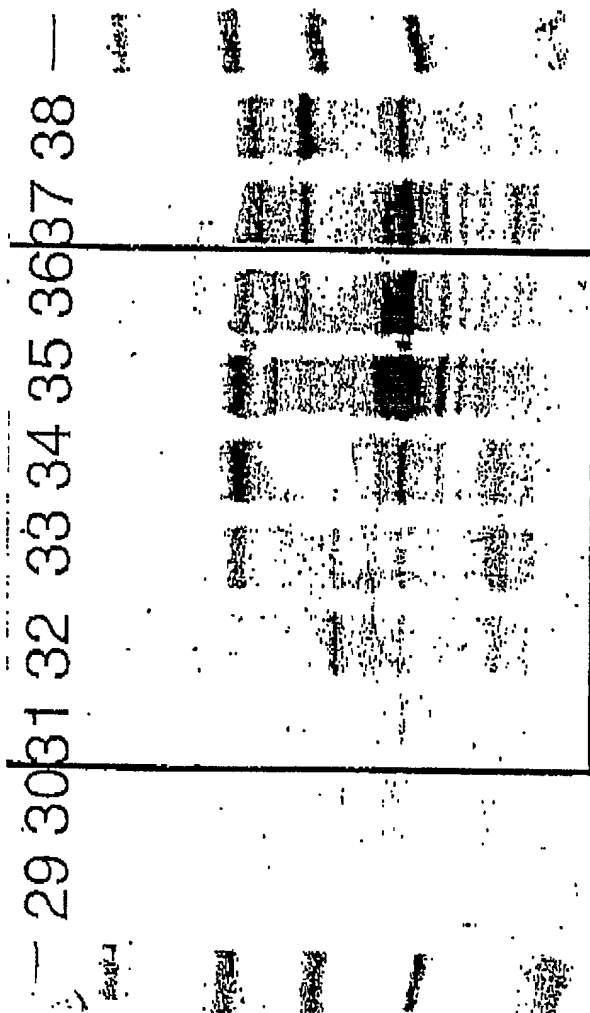


FIGURE 12

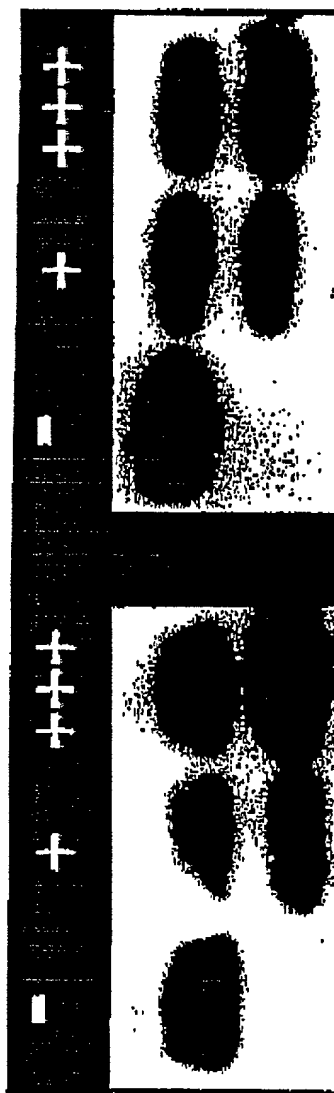


FIGURE 11

FIGURE 13A

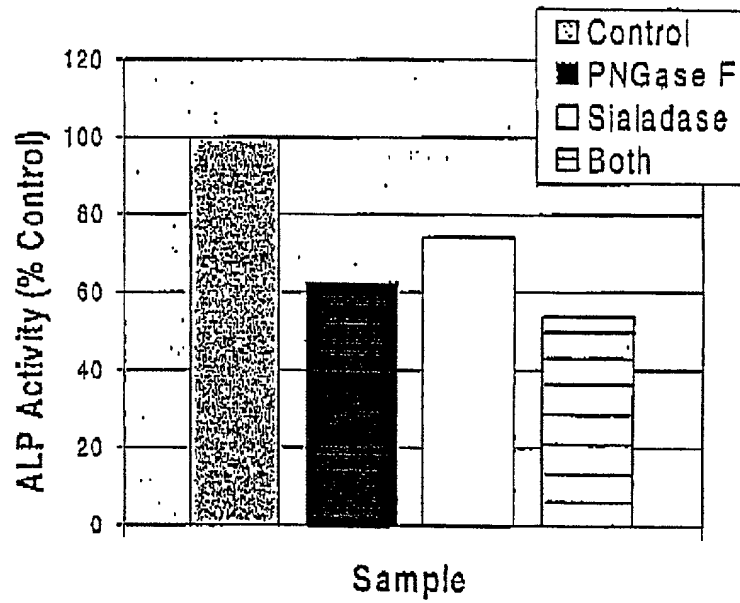
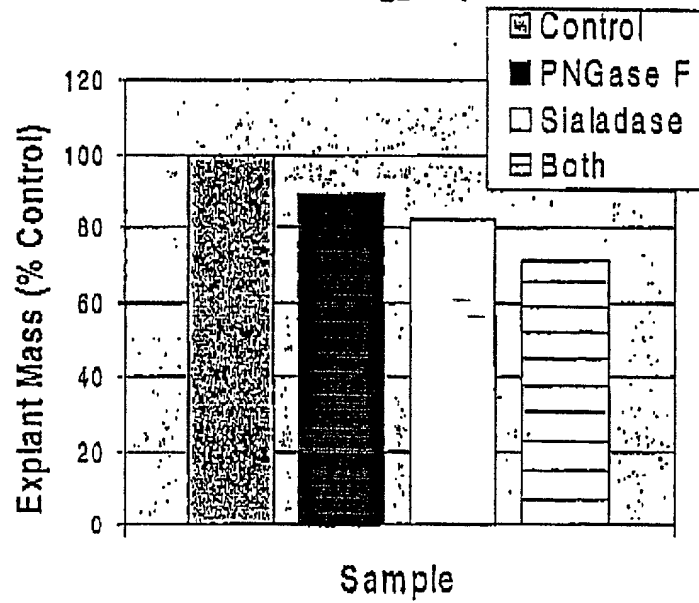


FIGURE 13B

Figure 14 Antibody Listing

Specificity	Antigen	Host Species	PC/MC	Source	Catalog No.
TGF- β 1 (human)	Protein	Rabbit	Polyclonal	Promega	G1221
TGF- β 2 (human)	Peptide	Rabbit	Polyclonal	Santa Cruz Biotechnology	sc-90
TGF- β 3 (human)	Peptide	Rabbit	Polyclonal	Santa Cruz Biotechnology	sc-82
BMP-2 (human)	Protein	Rabbit	Polyclonal	Austral Biologics	PA-513-9
BMP-3 (human)	Peptide	Chicken	Polyclonal	Research Genetics	NA
BMP-4 (human)	Peptide	Goat	Polyclonal	Santa Cruz Biotechnology	sc-6896
BMP-5 (human)	Peptide	Goat	Polyclonal	Santa Cruz Biotechnology	sc-7405
BMP-6 (human)	Peptide	Mouse	Monoclonal	Novocastri Laboratories	NCL-BMP6
BMP-7 (human)	Peptide	Rabbit	Polyclonal	Research Genetics	NA
TGF- β 1 (human)	Peptide	Goat	Polyclonal	Santa Cruz Biotechnology	sc-1884
osteocalcin (bovine)	Protein	Mouse	Monoclonal	DSHB	AON-1
osteocalcin (bovine)	Protein	Rabbit	Polyclonal	Accurate Chemicals	A761/R111
serum albumin (bovine)	Protein	Rabbit	Polyclonal	Chemicon International	AB870
transferrin (human)	Protein	Chicken	Polyclonal	Chemicon International	AB797
apo-A1 lipoprotein (human)	Protein	Goat	Polyclonal	Chemicon International	AB740

Figure 15A Identification of Proteins by Amino Acid Sequencing of Tryptic Fragments from 1D Gels

Band	Sample	Sequence Data	Best Database Match	Match	Identification	Species	Acc. No.	AAs
1								
2	fx 49 (1579)	XLAAGYDVEK	ALAAAGYDVEK	11/11	histone H1.c	human	67668 (NCBI)	65-75
3	fx 67 (1346)	SLEKVCADLR	SLEKVCADLR	11/11	40s Ribosomal Protein S20	rat	R3RT20 (PIR)	31-41
4	fx 65 0	(V)VCGMLGFPSEAPV	VVCGMLGFPGEKRV	11/14	LORP	mouse	AAC95338 (NCBI)	213- 228
5	N terminal seq	STGVLLPLQNNELPG	STGVLLPLQNNELPG	15/15	BMP-3	human	4557371 (NCBI)	290- 304
	fx 72 (3925)	STGVLLPLQNNELPGA EYQY	STGVLLPLQNNELPGA AEYQY	20/20	BMP-3	human	4557371 (NCBI)	290- 309
	fx 74 (3409)	STGVLLPLQ	STGVLLPLQ	9/9	BMP-3	human	4557371 (NCBI)	290- 298
6	fx 55 (1566)	(S)QTLOFXE	SQTLOFDE	7/8	BMP-3	human	4557371 (NCBI)	346- 353
	fx 47	NYAF	no match		???			
	N terminal seq	HAGKYSREKNT(P)A(P)	HGGKYSREKKNQPKP	11/14	α 2-Macroglobulin Receptor Assoc. Pro.	human	P30533 (Swiss-Prot)	31-46
	fx 57 (1438)	SQTLOFDEQ	SQTLOFDEQ	9/9	BMP-3	human	4557371 (NCBI)	346- 354
	fx 57 (1852)	SLKPSNHA	SLKPSNHA	8/8	BMP-3	human	4557371 (NCBI)	410- 417
7	fx 51 (1093)	AALRPLVKP	AALRPLVKP	9/9	60s Ribosomal Protein L32	mouse	P17832 (Swiss-Prot)	1-8
	fx 37 (no MS)	A(I)(Q)VERYV	AIVER	5/5	60s Ribosomal Protein L32	mouse	P17832 (Swiss-Prot)	109- 113
	fx 37 (no MS)	A(I)(Q)VERYV	HQSDRYV	5/7	60s Ribosomal Protein L32	mouse	P17832 (Swiss-Prot)	22-28
8	fx 78 0	XALF(G)AQLGXALGPI	no match		???			
9	fx 56 (1587)	SQTLOFDEQT	SQTLOFDEQT	10/10	BMP-3	human	P12645 (Swiss-Prot)	346- 355

Figure 15B Identification of Proteins by Amino Acid Sequencing of Tryptic Fragments from 1D Gels

Band	Sample	Sequence Data	Best Database Match	Match	Identification	Species	Acc. No.	AAs
11	fx 55 (1311)	SQTLXF	SQTLQF	5/6	BMP-3	human	4557371 (NCBI)	346-351
	fx 47 (1772)	VLATVTKPVGGDK	VLATVTKPVGGDK	13/13	60s Ribosomal Protein L6	human	Q02878 (Swiss-Prot)	67-99
	fx 76 (1795)	xVFAL	VFAL	4/4	60s Ribosomal Protein L6	human	Q02878 (Swiss-Prot)	273-276
	fx 51 (1145)	AVPQLQGYLR	AVPQLQGYLR	9/10	60s Ribosomal Protein L6	human	Q02878 (Swiss-Prot)	252-271
18								
22	fx 58 (1101)	ALDAAYCFR	ALDAAYCFR	9/9	TGF- β 2	human	P08112 (Swiss-Prot)	303-311
	fx 69 (no match)	GYNANFCAGACPVL	GYNANFCAGACPVL	14/14	TGF- β 2	human	P08112 (Swiss-Prot)	340-353
	fx 66 (1411.71)	VNSQSLSPY	VNSQSLSPY	9/9	SPP24	bovine	Q27967 (Swiss-Prot)	42-50
25	fx 39 (1470)	KAAKPSV(P)	KAAKPSVP	8/8	Histone H1.x	human	JC4928 (PIR)	199-206
28								

fx = fraction number (molecular weight of fragment, as measured by SDS-PAGE)

Figure 4A Identification of Proteins by Mass Spectrometry of Tryptic Fragments from 1D Gels

Band	Mass Spec Profile	Species	Acc. No.	Mass Spec Data	Mass Spec Database	Mass Difference	AAS	% Coverage	Comments
1	4 peaks match with histone H1.c	human	87668 (NCBI)	1172.97	1172.37	0.60	110-121	22	15 MS peaks match with Band 2
				1579.87	1579.71	0.16	65-78		
				1708.47	1707.89	0.58	64-78		
				2011.58	2012.32	-0.74	35-54		
2	3 peaks match with histone H1.c	human	87668 (NCBI)	1579.76	1579.71	0.05	65-79	16	identification of stained peptide confirmed by sequence analysis
				1708.02	1707.99	0.03	64-79		
				2012.12	2012.32	-0.20	35-54		
				1129.76	1129.40	0.36	50-59	62	
3	7 peaks match with ribosome S20	rat	R3R120 (PIR)	1156.21	1155.30	-0.09	75-83		
				1334.46	1334.62	-0.16	58-66		
				1352.13	1351.58	0.55	88-99		
				1518.04	1517.77	0.27	9-21		
				1919.02	1919.19	-0.17	5-21		
				3404.02	3404.87	-0.85	88-119		
4	3 peaks match with Lysyl Oxidase Rp	human	NP002309 (Swiss-Prot)	1987.95	1988.27	-0.32	150-167	8	12 MS peaks match with Band 8
				2410.35	2410.63	-0.28	648-669		
				2610.57	2610.10	0.47	455-478		

Figure 16B Identification of Proteins by Mass Spectrometry of Tryptic Fragments from 1D Gels

Band	Mass Spec Profile	Species	Acc. No.	Mass Spec Data	Mass Spec Database	Mass Difference	AAs	% Coverage	Comments
5	9 peaks match with BMP-3	human	4557371 (NCBI)	1113.32	1113.31	0.01	361-368	48	% coverage calculation is relative to the mature BMP-3, 183 AAs (290-472)
				1436.53	1438.58	-0.05	346-357		
				1566.76	1566.76	0.00	345-357		
				1651.98	1651.91	-0.05	410-424		
				1794.09	1794.02	0.07	346-360		
				2268.46	2268.63	-0.17	374-392		
				2424.45	2424.81	-0.36	373-392		
6	3 peaks match with $\alpha 2$ -Macroglobulin RAP	human	P30533 (Swiss-Prot)	3409.15	3407.77	1.38	290-318	17	Identification of stained peptide confirmed by sequence analysis
				1002.24	1002.15	0.09	283-290		
				2362.58	2362.43	0.15	129-150		
				3048.51	3048.52	-0.01	257-282		
				1566.93	1566.75	0.18	346-357		
	2 peaks match with BMP-3	human	4557371 (NCBI)	1651.88	1651.81	-0.03	410-424	15	% coverage calculation is relative to the mature BMP-3, 183 AAs (290-472)

Figure 16C Identification of Proteins by Mass Spectrometry of Tryptic Fragments from 1D Gels

Band	Mass Spec Profile	Species	Acc. No.	Mass Spec Data	Mass Spec Database	Mass Difference	AAs	% Coverage	Comments
7	4 peaks match with ribosone L32	mouse	P17932 (Swiss-Prot)	1033.25	1033.17	0.08	67-75	33	
				1093.31	1093.40	-0.09	1-10*		
				1134.72	1134.28	0.44	65-74		
				1448.78	1449.68	0.12	19-28		
				1060.42	1060.20	0.22	102-111	21	% coverage calculation is relative to the mature BMP-3, 163 AAs (290-472)
8	1 peak matches with Lysyl Oxidase RP	human	NP002309 (Swiss-Prot)	1113.39	1113.31	0.08	361-368	3	12 MS peaks match with Band 4
				1360.26	1360.58	-0.32	190-200		
				1652.28	1651.91	0.37	410-424		
				1793.62	1794.02	-0.40	346-360		
				2410.37	2410.63	-0.26	648-669		
9	5 peaks match with BMP-3	human	4557371 (NCBI)	1113.14	1113.31	-0.17	361-368	36	% coverage calculation is relative to the mature BMP-3, 163 AAs (290-472)
				1438.60	1438.58	0.02	346-357		
				1566.77	1566.76	0.01	345-357		
				1651.91	1651.61	0.30	410-424		
				2901.67	2901.19	0.48	41-66		
				3408.94	3407.77	1.17	290-316		

Figure 1cD Identification of Proteins by Mass Spectrometry of Tryptic Fragments from 1D Gels

Band	Mass Spec Profile	Species	Acc. No.	Mass Spec Data	Mass Spec Database	Mass Difference	AAs	% Coverage	Comments
11	5 peaks match with BMP-3	human	4557371 (NCBI)	1113.23	1113.31	-0.08	361-368	48	% coverage calculation is relative to the mature BMP-3, 183 AAs (290-472)
				1651.73	1651.91	-0.18	410-424		
				1793.58	1794.02	-0.44	346-360		
				2424.24	2424.81	-0.57	373-392		
				3408.34	3407.77	0.57	280-318		
18	5 peaks match with ribosoma L6	human	Q02878 (Swiss-Prot)	1140.38	1140.23	0.15	114-122	18	
				1526.88	1526.86	0.02	141-155		
				1059.15	1059.12	0.03	10-20		
				1145.36	1145.35	0.01	262-271		
				1386.74	1386.66	0.08	260-271		
	4 peaks match with TGF-β2	human	P08172 (Swiss-Prot)	1101.20	1101.26	-0.06	303-311	52	
				1175.26	1175.42	-0.16	400-409		
				2240.37	2240.60	-0.23	312-328		
				2691.70	2691.91	-0.21	340-352		
				1410.93	1411.60	-0.67	42-53		
	5 peaks match with SPP24	bovine	Q27967 (Swiss-Prot)	1447.59	1447.65	-0.06	113-124	30	
				1540.84	1540.80	0.04	86-98		
				1868.10	1869.05	0.95	62-77		
				2268.47	2268.57	-0.10	33-53		

Figure. 16E Identification of Proteins by Mass Spectrometry of Tryptic Fragments from ID Gels

Band	Mass Spec Profile	Species	Acc. No.	Mass Spec Data	Mass Spec Database	Mass Difference	AAs	% Coverage	Comments
22	5 peaks match with TGF- β 2	human	P08112 (Swiss-Prot)	1101.15	1101.26	-0.11	303-311	53	
				1175.13	1175.42	-0.29	400-409		
				2084.16	2084.42	-0.26	312-347		
				2240.25	2240.60	-0.35	312-328		
				2691.61	2691.91	-0.30	340-362		
	2 peaks match with SPP24	bovine	Q27967 (Swiss-Prot)	1411.23	1411.60	-0.37	42-53	11	
25	5 peaks match with histone H1.x	human	JC4928 (PIR)	1447.40	1447.65	-0.25	113-124	14	
				1208.46	1208.40	0.06	48-57		
				1221.71	1222.35	-0.64	107-118		
				1349.65	1350.52	-0.67	107-119		
	5 peaks match with BMP-3	human	4557371 (NCBI)	1384.57	1384.59	-0.02	48-58		
				1732.23	1732.97	-0.74	43-57		
				1060.43	1060.20	0.23	102-111	31	% coverage calculation is relative to the mature BMP-3, 183 AAs (290-472)
				1438.83	1438.58	0.25	346-357		
				1566.92	1568.76	0.16	345-357		
				1651.80	1651.91	-0.11	410-424		
				3408.86	3407.71	1.09	290-318		

Figure 16F Identification of Proteins by Mass Spectrometry of Tryptic Fragments from 1D Gels

Band	Mass Spec Profile	Species	Acc. No.	Mass Spec Data	Mass Spec Database	Mass Difference	AAS	% Coverage	Comments
29	4 peaks match with BMP-3	human	4557371 (NCBI)	1113.72	1113.31	-0.09	301-368	27	% coverage calculation is relative to the mature BMP-3, 183 AAS (29D-472)
				1438.70	1438.58	0.12	346-357		
				1566.88	1566.75	0.11	345-357		
				3409.04	3407.77	1.27	290-318		

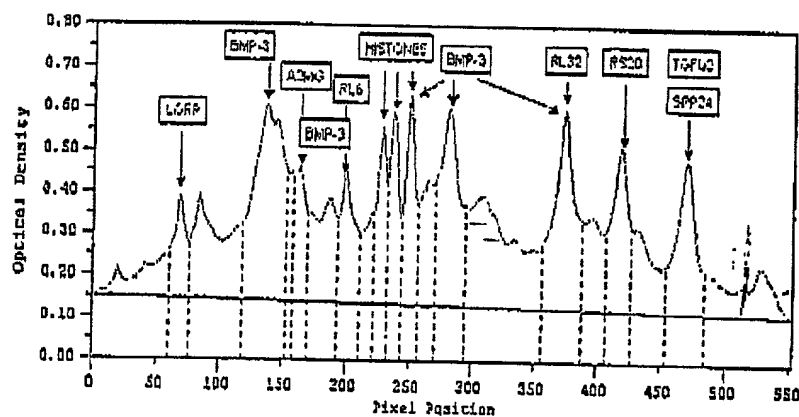


FIGURE 17A

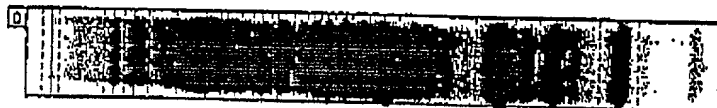


FIGURE 17B

Figure 18 Quantitation of Identified BP proteins

Identified Protein	Percentage of Total Protein
LORP	2
BMP-3	11
BMP-3 & A2-MG	3
RL6 & BMP-3	4
Histone	3
Histone	3
Histone & BMP-3	4
BMP-3	8
RL32 & BMP-3	8
RS20	5
SPP24 & TGF- β 2	6
Total	56%

003327-8604160

Figure 19A Identification of Proteinby Mass Spectrometry of Fragments from 2D Gels

Spot	Digest	Mass Spec Profile	Species	Acc. No.	MS Peaks			AAs	% Coverage	Comments
					Data	Database	Diff			
1	Lys-C	2 peaks match with Coagulation Factor XIIIb	Human	P05160 (Swiss-Prot)	1837.01	1837.14	-0.13	472-487	8	peptide match confirmed by sequence analysis
					1921.65	1921.14	0.51	368-382		
					2679.51	N/A	N/A	488-504		
2	Trypsin	2 peaks match with LORP	Human	NP002309 (Swiss-Prot)	1609.57	1609.86	-0.31	241-253	5	
					2410.89	2410.63	0.26	648-669		
3	Lys-C	8 peaks match with Cathepsin L Precursor	Bovine	P25975 (Swiss-Prot)	1487.26	1486.60	0.66	105-116	41	
					1546.84	1546.70	0.14	58-70		
					1861.16	1860.80	0.36	21-33		
					1881.25	1880.80	1.06	301-314		
					1834.71	1834.60	0.71	318-334		
					2352.90	2351.50	1.40	274-285		
					2381.50	2380.70	0.80	239-261		
					2721.51	2721.10	0.41	131-154		

Figure 1/C Identification of Proteins by Mass Spectrometry of Fragments from 2D Gels

Spot	Digest	Mass Spec Profile	Species	Acc. No.	MS peaks			AAs	% Coverage	Comments
					Data	Database	Diff			
7	Lys-C	4 peaks match with TGF- β 2	Bovine	P21214 (Swiss-Prot)	774.56	774.80	-0.34	26-31	42	
					808.69	809.94	-0.25	32-37		
					1175.12	1175.43	-0.31	98-107		
					3168.10	3166.66	1.44	1-25		
		1 peak matches with SPP24	Bovine	Q27967 (Swiss-Prot)	2187.77	2187.51	0.26	42-60	10	
8	Trypsin	12 peaks match with ribosome L3	Bovine	P39872 (Swiss-Prot)	917.39	917.14	0.25	348-355	37	
					984.23	984.15	0.08	10-18		
					1192.62	1192.40	0.22	286-296		
					1380.87	1380.65	0.02	249-260		
					1484.80	1484.63	0.17	103-114		
					1620.86	1620.82	0.04	103-115		
					1778.84	1770.00	-0.16	34-49		
					2238.43	2230.55	-0.12	30-49		
					2325.99	2325.65	0.34	177-197		
					2661.31	2661.04	0.27	200-223		
					2897.94	2898.43	-0.49	70-98		
					2946.10	2948.35	-0.25	198-223		

Figure 10 Identification of Proteins by Mass Spectrometry of Fragments from 2D Gels

Spot	Digest	Mass Spec Profile	Species	Acc. No.	MS Peaks			AAs	% Coverage	Comments
					Data	Database	Diff			
9	Trypsin	7 peaks match with ribosome S3a	Mouse	P97351 (Swiss-Prot)	920.05	920.10	-0.05	19-26	29	
					1218.29	1218.31	-0.02	152-161		
					1348.62	1348.49	0.13	151-161		
					1516.69	1516.69	0.00	174-185		
					1593.72	1593.82	-0.10	94-108		
					1719.91	1720.00	-0.09	199-212		
					1953.12	1953.16	-0.04	65-81		
10	Trypsin	4 peaks match with histone H1.c	Human	87668 (NCBI)	1327.75	1327.58	0.19	34-46	23	
					1579.70	1579.71	-0.01	65-79		
					1707.65	1707.89	-0.24	64-79		
					2147.17	2147.53	-0.36	1-21		
11	Trypsin	6 peaks match with ribosome S4	Human	P12750 (Swiss-Prot)	1168.48	1168.38	0.10	230-239	23	
					1216.39	1216.39	0.00	134-144		
					1354.03	1353.61	0.42	230-241		
					1507.81	1507.88	-0.07	198-210		
					1557.75	1557.98	-0.23	37-48		
					2140.34	2140.58	-0.24	221-239		
					2591.80	2591.90	-0.10	77-98		